Universidade de Lisboa

Faculdade de Farmácia

Investigation of the Release of Colonic Delivery Marketed Products

Alexandra Isabel Rodrigues Palma Vaz

Mestrado Integrado em Ciências Farmacêuticas

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Tese de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

A mesalazina é um fármaco anti-inflamatório utilizado como terapia de primeira linha para a induzir e manter a remissão da colite ulcerosa e da doença de Crohn nos estadios iniciais da doença, atuando localmente nos locais inflamados do trato gastrointestinal. Assim, os principais fatores determinantes na resposta clínica irão ser a libertação do fármaco e a sua biodisponibilidade no local de ação. O presente trabalho tem como objetivo prever o local e a extensão da libertação *in vivo* da mesalazina após administração oral de vários medicamentos de libertação modificada. Com este objetivo, foram desenvolvidos testes *in vitro* que refletem os vários ambientes pelos quais uma forma farmacêutica com mesalazina incorporada é exposta durante a sua passagem pelo trato gastrointestinal. Foi utilizado o aparelho de dissolução FE tipo I, bem como recipientes de plástico com agitação horizontal contendo os meios descritos na FE 9 para simular a passagem por todo o trato gastrointestinal sob diferentes condições fisiológicas. Todos os medicamentos testados estão disponíveis no mercado alemão. Apesar de todos os medicamentos serem indicados para os mesmos objetivos terapêuticos, cada um dos mesmos apresentou um perfil de dissolução distinto. Os resultados dos ensaios de dissolução para ambos os comprimidos de Asacol indicaram que a libertação de mesalazina deve ocorrer na zona distal do trato gastrointestinal, uma vez que a libertação do fármaco é desencadeada por pH mais alto. Os comprimidos de Claversal e o Salofalk mostraram uma libertação semelhante à de uma libertação pulsátil. Para ambas as formulações, espera-se que a libertação de mesalazina ocorra na proximidade do intestinal delgado. Os *pellets* de Claversal e os comprimidos de Mezavant exibiram características de libertação retardada e libertação prolongada. Ambas as formas farmacêuticas de Pentasa libertaram cerca de 50% do fármaco no estômago do modelo, em linha com a ausência de revestimento entérico nestas formas farmacêuticas. Desta forma, a libertação do fármaco pelo Pentasa dependerá do tempo de residência gástrica. Como os doentes com doenças inflamatórias intestinais apresentam locais inflamados diferentes e, como os aspetos fisiológicos podem variar entre doentes, as abordagens descritas aqui são úteis na tomada de decisão quanto ao medicamento mais adequado para cada doente.

Palavras-chave: 5-ASA; administração colónica; revestimento gastro-resistente; mesalazina.

Abstract

Mesalazine is an anti-inflammatory drug regarded as the first-line therapy to induce and maintain the remission of UC and CD in the early stages of the disease. This API is intended to act locally at the inflamed sites of the GI tract. Thus, the major factors determining the clinical response are the time and location at the site of action of the drug. The present work aims to predict the site and extent of *in vivo* mesalazine release after oral administration of several commercial modified-release medicines. With this purpose, *in vitro* tests were developed reflecting the changing environment that a dosage form incorporating mesalazine is exposed to as it moves through the GI tract. The EP dissolution apparatus type I was used, as well as plastic containers shaken horizontally. Compendial media were used to simulate the passage throughout the GI tract under different physiological conditions. All tested medicines were available on the German market. Even though all medicines were indicated for the same therapeutic objectives, each of the medicines displayed a distinctive dissolution profile. The results of the dissolution tests for both Asacol tablets indicated that mesalazine release is likely to occur in the distal parts of the GI tract, since their drug release is triggered by higher pH levels. Claversal tablets and Salofalk showed a similar burst-like release. For both medicines, mesalazine release is expected to occur near the proximal portion of the small intestine. Claversal pellets and Mezavant tablets displayed delayed release and extended release characteristics. Both Pentasa dosage forms released about 50% of their drug load in the stomach compartment of the model. This is attributed to the absence of an enteric coating in these dosage forms. Therefore, the release of the drug by Pentasa in the colon will be dependent on gastric residence time. As IBD patients have different distribution patterns of inflamed sites, and the physiology vary between patients, the findings of this work are expected to help on making a decision on which medicine fills the needs of each patient.

Keywords: 5-ASA; colonic delivery; enteric-coating; mesalazine.

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Abbreviations

API – Active Pharmaceutical Ingredient

CD - Crohn's disease

EC – Ethyl cellulose

GI – Gastrointestinal

HPMC - Hydroxypropyl methylcellulose

IBD – Inflammatory Bowel Disease

PEG – Poly (ethylene glycol)

PMA - Poly (methacrylic acid)

UC – Ulcerative colitis

UV – Ultraviolet

Vis - Visible

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Chapter 1 – Introduction

1.1. Colonic Drug Delivery System

Paul Ehrlich (1854-1915), a German physician and a scientist, was the first person designing the concept of a drug targeting a specific agent in order to maximize the therapeutic efficacy with a simultaneous reduction of side effects. To this achievement, Ehrlich called it the “magic bullet concept”. This attainment was obtained from his experiences in the treatment of infectious diseases with drugs derived from the German dye industry (1). However, more than a hundred years later, this concept of a drug that goes straight to its intended target can be extend to other areas of research such as cancerous tumours and autoimmune diseases (2). This is mainly due to new insights regarding the pathophysiology of such diseases, making also possible the development of innovative drug carrier systems such as a colonic drug delivery system.

Colonic Drug Delivery System is part of a much broader classification called “modified release drug delivery system”. This type of drug delivery system is characterized by the ability to modulate the apparent absorption (by altering the rate of release of the drug substance) and/or vary the place of release of the active substance(s), in order to attain specific clinical objectives not achievable by conventional or immediate release dosage forms (3,4). More specifically, modified release oral dosage forms include extended release, also known as “sustained release”, “prolonged release” and “controlled release”, “pulsatile release” and “delayed release” (4). An extended release dosage form intends to make the drug available over a prolonged period after ingestion, which leads to a reduction in dosing frequency compared to a drug presented in a conventional dosage form. This results in a more constant and prolonged therapeutic effect and also in an improved patient compliance (5). Regarding pulsatile release dosage form, this type of modified release dosage form releases a portion of the total payload in a burst followed by periods of little to no release (lag phase) in a defined temporal pattern. This drug delivery system brings some clinical benefits such as optimization of chronotherapy (6), it allows to mimic natural patterns of endogenous secretion (7) and also provides optimal therapy for tolerance-inducing drugs where constant levels cause receptor down-regulation (8). Finally, delayed release dosage forms are characterized by releasing the active substance(s) at a time other than immediately (4). This type of drug delivery system not only includes enteric release, where the drug release is delayed until it has passed through the stomach, but also

colonic release, where the drug is delivered in the colonic region of the gastrointestinal (GI) tract (3).

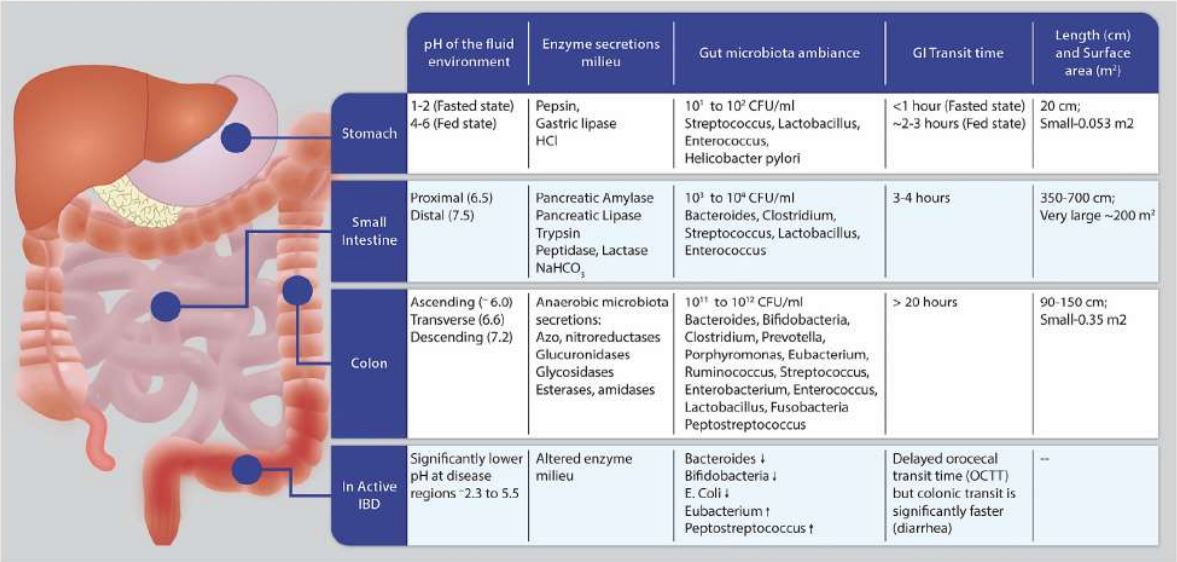
Colonic Drug Delivery System has been considered in recent years as a quite promising drug delivery system due to its usefulness for delivering a variety of therapeutic agents locally in numerous colonic pathological conditions (9–11). The optimal site for delivery in the colon is considered to be the proximal or ascendant colon (12,13). The development of this type of drug delivery system emerged from the need of having a suitable therapeutic concentration in the damaged colon area with reduced systemic side effects. Furthermore, the fact that the conventional oral release dosage forms were not suitable for targeting drugs to the lower GI tract also promoted the development of colonic drug delivery systems (14).

Therapeutics, which include drugs, proteins and peptides, are administrated either orally (enteric coated pills, tablets and capsules), or parenterally (intravenous and subcutaneous injections) or rectally (enemas, suppositories and foams) (15). The oral route, which is the main focus of this work, is the preferable choice since it presents many benefits, namely an inexpensive production, improved patient adherence, accurate dosing, an excellent stability and storability of dosage forms (2,16) and the fact that possible degradation at the site of administration is reduced (17).

Moreover, aside from the dosage form, it also must be taken into consideration other aspects that can influence the success of the colonic drug delivery system. These aspects include the fact that colon is located in the distal part of the GI tract, the properties of the drug and the anatomy and physiology of colon in healthy and pathological conditions. Regarding drug properties, the two main properties that we must take into account are the stability and the solubility of the drug. Since the drug is in contact with the colonic content *e.g.*, dietary residues, intestinal secretions, mucus, or faecal matter, non-specific interactions may occur leading to a negative influence on the stability of the drug (18). In addition, the colonic bacterial enzymes may also degrade the drug, rendering it ineffective. The other property, which is drug solubility, can also be altered. Due to low colonic luminal fluid volume, higher viscosity, and a neutral pH in colon, the solubilization of the drug could be a rate-limiting factor for the colonic absorption (16). Furthermore, some anatomical/physiological factors may also influence the formulation/development of a colon-specific drug delivery system.

The human large intestine is approximately 1.5 m long and is divided in colon (ascending, transverse and descending) and in a small distal part called rectum. The physiology and the physical properties of the colonic contents can differ in each region of colon (Figure 1). Also, there is variability in movement of food and dosage forms

across the colon, which can be a challenge in the development of colonic drug delivery systems (19).



	pH of the fluid environment	Enzyme secretions milieu	Gut microbiota ambiance	GI Transit time	Length (cm) and Surface area (m^2)
Stomach	1-2 (Fasted state) 4-6 (Fed state)	Pepsin, Gastric lipase HCl	10^1 to 10^2 CFU/ml Streptococcus, Lactobacillus, Enterococcus, Helicobacter pylori	<1 hour (Fasted state) ~2-3 hours (Fed state)	20 cm; Small-0.053 m2
Small Intestine	Proximal (6.5) Distal (7.5)	Pancreatic Amylase Pancreatic Lipase Trypsin Peptidase, Lactase $NaHCO_3$	10^3 to 10^4 CFU/ml Bacteroides, Clostridium, Streptococcus, Lactobacillus, Enterococcus	3-4 hours	350-700 cm; Very large ~200 m^2
Colon	Ascending (~ 6.0) Transverse (6.6) Descending (7.2)	Anaerobic microbiota secretions: Azo, nitroreductases Glucuronidases Glycosidases Esterases, amidases	10^{11} to 10^{12} CFU/ml Bacteroides, Bifidobacteria, Clostridium, Prevotella, Porphyromonas, Eubacterium, Ruminococcus, Streptococcus, Enterobacterium, Enterococcus, Lactobacillus, Fusobacteria Peptostreptococcus	> 20 hours	90-150 cm; Small-0.35 m2
In Active IBD	Significantly lower pH at disease regions ~2.3 to 5.5	Altered enzyme milieu	Bacteroides ↓ Bifidobacteria ↓ E. Coli ↓ Eubacterium ↑ Peptostreptococcus ↑	Delayed orocecal transit time (OCTT) but colonic transit is significantly faster (diarrhea)	--

Figure 1. Anatomical and physiological characteristics of various segments of GI tract and in inflamed colon region. Adapted from (15)

Another physiological factor that affects colonic drug delivery is the variation of pH values in the GI tract. There is substantial intra- and inter-subject variability in the pH of the GI tract between pathological conditions (such as inflammatory bowel disease as ulcerative colitis (UC) and Crohn's disease(CD)), fasted/fed states, gender and ages in humans (20,21). Furthermore, the pH in colon can also be influenced by external factors such as a carbohydrate rich diet (22) or the use of polysaccharide-based drugs *e.g.,* laxative drugs (23). These pH changes in GI tract can be a suitable parameter to deliver therapeutics to specific regions. However, since pH values tend to vary with different aspects, relying on the dynamic pH of GI tract must be evaluated carefully in order to provide enough targetability.

Gastric emptying and transit time also play an important role in the performance of colonic drug delivery systems. The normal gastric emptying takes place within 2 h and the colonic arrival occurs after 5 h (24). However, some pathological conditions such as diarrhoea, constipation, UC and CD, can influence these physiological characteristics. Diarrhoea patients usually present shorter transit time whereas constipation patients have longer transit times. Regarding patients with UC, they are known to have shorter colonic times (around 24 h) compared to healthy subjects (around 52 h) (25). Furthermore, the GI transit time varies between subjects depending on other factors such as diet, mobility and stress. Also, it was already described that smaller particles have a longer transit time than larger particles (26). Thus, due to all these aspects that can vary,

one should be very cautious when designing colonic drug delivery systems based on time.

Moreover, the colon is known to contain over 400 different species of aerobic and anaerobic microorganisms (27). This specific niche forms a barrier against invasive pathogenic bacteria and helps in the development of the intestinal immune system (28,29). Furthermore, the intestinal microbiota plays an important role in maintaining the GI physiology and provides a benefit to the host in the breakdown of indigestible food. The latter occurs because these bacteria contain several hydrolytic and reductive metabolizing enzymes (30), capable of catalysing a wide range of reactions (31). However, the drug intake (especially, antibiotics and laxatives) and diet style can significantly alter the microbiota-enzyme secretions, due to changes in microbiome composition. In these cases, the release of some therapeutics whose polymers are degraded enzymatically by colonic bacteria can be altered. In this way, colonic drug delivery systems depending on colon microbiome must take into consideration all the above mentioned.

Thus, taking into consideration all the features of GI tract above mentioned, one can summarize all colonic drug delivery systems into two main groups: time-dependent or site-specific systems (3,32).

1.1.1. Time-Dependent Drug Delivery Systems

A majority of the marketed colonic delivery products are timed release systems. This group of drug delivery systems is influenced not only by the system itself (32) but also by the transit times of the GI tract (3,17,33). In this approach, the carriers or polymers used to control the drug release intend to retard it until the delivery system reaches the colon. To this end, one can select a surface erosion system, a drug delivery system containing a core capable of expand or an osmotically controlled drug delivery system. The polymers used can be part of matrix dosage forms or work as a coating layer on a single unit or multi-particulate delivery systems. In these approaches, a multi-particulate drug delivery system, such as pellets, offers some benefits when compared with single unit dosage forms, as tablets. The reduced size of multi-particulates drug delivery systems (approximately, 1 mm) allows their continuous and unrestricted transit through the GI tract, suffering less influence by intestinal transit and gastric emptying (34,35). Furthermore, pellets diffuse slowly over a wide area of the colon and they are retained longer in the ascending colon than tablets (36–38). This can be beneficial for inflammatory bowel disease (IBD) patients, where local concentrations at the sites of inflammation are needed. Moreover, multi-particulate dosage forms can be widely and

uniformly dispersed throughout the GI tract due to their small size promoting a more uniform and safe drug absorption (35,39–41). Finally, the release failure of one pellet does not compromise the total release behaviour due to the multiple units of the multi-particulate drug delivery system (35,40,42). Therefore, due to all the disadvantages of single unit dosage forms over multi-particulate drug delivery systems, the choice for a tablet in this approach often requires an additional enteric coating when the objective is colonic drug delivery.

1.1.1.1. Surface Erosion Systems

In a surface erosion system, the polymers degradation starts to occur at the scaffold surface, from the exterior to the inner core (Figure 2) (43,44). When the purpose is the delivery to the colon, swellable hydrophilic polymeric materials are commonly used. Especially, cellulose ethers, such as hydroxypropyl methylcellulose (HPMC), hydroxypopylcellulose (HPC) and hydroxyethylcellulose (HEC), are extensively employed due to their safety profile and affordability. When these polymers are exposed to aqueous biological fluids, they swell, dissolve and suffer mechanical erosion phenomena, resulting in a delay of drug release from the core of the dosage form (45). The erosion kinetics (and consequently drug release) is controllable and reproducible making this approach suitable for many drug delivery applications. Furthermore, this approach is appropriate for water-vulnerable drugs since the water permeation rate is slow (43,44).

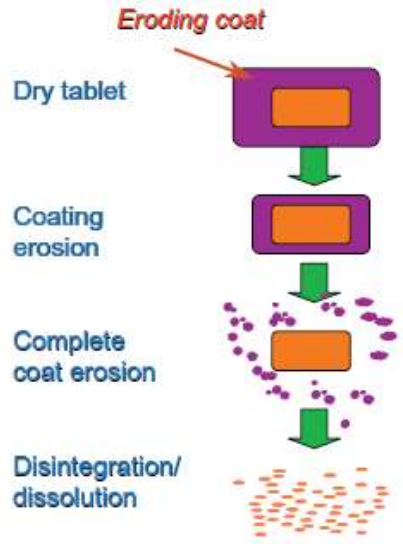


Figure 2. Schematic representation of a surface erosion system. Adapted from (3)

Steed *et al*. (46) developed an enteric-coated "TIME CLOCK" delivery device aiming for mesalazine to reach the colon. The TIME CLOCK system consists of a tablet

core coated with a time-dependent polymer, namely HPMC. The lag time for drug release is dependent on the coating thickness (20%, 35% and 50%). Results from pharmacoscintigraphic studies performed in healthy subjects demonstrated that this approach is appropriate for colon-targeted delivery of mesalazine, especially the 35% coating tablet. Thus, data indicate that the TIME CLOCK technology is efficient for the local treatment of IBD, since the 35% coating tablet delivers the drug in the proximal part of the colon (39,46).

1.1.1.2. Systems with Rupturable Polymeric Coats

Systems with rupturable polymeric coats for colon targeting contain a core formulation that is able to expand leading to a mechanical disruption of the coating and, consequently, to drug release. The polymeric film that coats the inner drug core is water-insoluble, but slightly permeable, when in contact with aqueous fluids. On the other hand, the core formulation swells due to osmotic or extremely water-swellable excipient-induced massive water uptake or, alternatively, it may result from the growth of gas carried out by effervescent additives (45) (Figure 3).

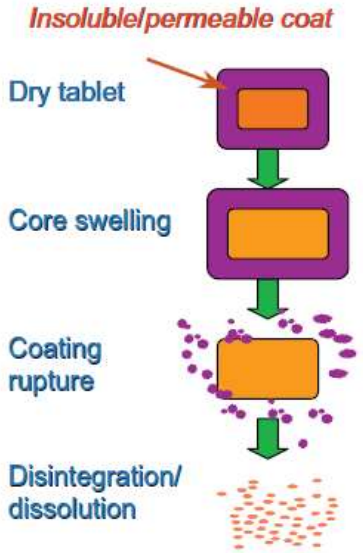


Figure 3. Schematic representation of a system with rupturable polymeric coats. Adapted from (3)

An example of this approach was developed by Fan et al. in which the release of diltiazem hydrochloride occurred after a lag time of 3 h in the small intestine (47). To this end, press-coated tablets containing a 1:2 mixture of ethyl cellulose (EC) and Eudragit L as the rupturable membrane and cross-linked polyvinylpyrrolidone in the inner core were investigated. It was proved that there is a direct relationship between the coat thickness and the lag time, whereas the drug release rate was considered independent of the coating level. Furthermore, a key step for the release of diltiazem hydrochloride was

dependent on the dissolution of Eudragit L in an environment of pH above 6 which caused the formation of pores within the EC membrane leading to water penetration into the core. This led to the swelling of the crosslinked polyvinylpyrrolidone resulting in the system disintegration. The *in vivo* studies also indicated to be in agreement with *in vitro* data. Thus, this approach may be suitable when the therapeutic effects of the drug are expected to occur several h after taking medicine, e.g., from midnight to dawn.

Another example of a system with rupturable polymeric coats is OROS-CT (Osmotic Release Oral System – Colon Targeted). This drug delivery system consists of a single osmotic unit or may include between 5 to 6 push-pull units encapsulated within a hard gelatin capsule (Figure 4) (48). Each push-pull unit contains an osmotic push layer and a drug layer, both covered by a semipermeable membrane. The semipermeable membrane allows the inward entry of water and GI fluids, but it is impermeable to the outward exit of the drug. Through the semipermeable membrane, there is an orifice thus allowing drug release. Eudragit S coats the semipermeable membrane surface to delay the drug release until the GI environment reaches pH≥7. Hence, water enters the unit causing the swelling of the osmotic push compartment and, therefore, forcing the drug out of the orifice. OROS-CT is designed to initiate drug release 3-4 h after it has left the stomach. This approach is capable of maintaining a constant release rate during 24 h in the colon (48). In this manner, OROS-CT allows a reduced dose frequency, an improved pharmacokinetic profile and an improved safety profile, resulting in an enhanced patient compliance and improved health outcomes (49,50).

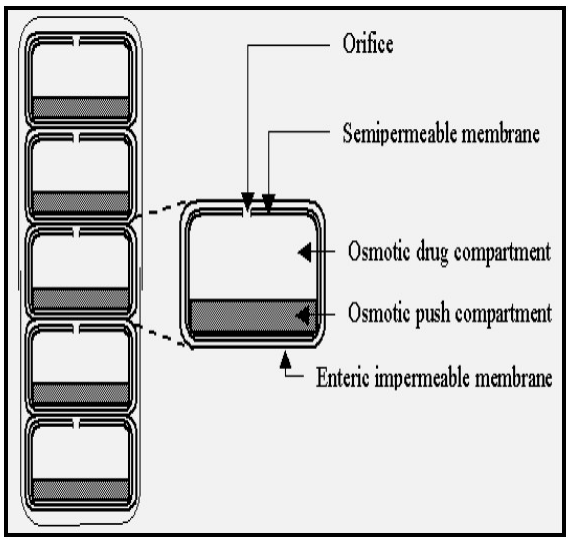


Figure 4. Schematic representation of the OROS-CT colon targeted drug delivery system. Adapted from (48)

1.1.2. Site-Specific Drug Delivery Systems

As previously explained in section 1.1, the success of drug delivery to the colon may rely on the exploitation of unique features of the colonic environment. The group of colonic drug delivery systems that take these features into consideration is called site-specific drug delivery systems. In this group, the triggering mechanism in the delivery system only reacts to the particular physiological conditions of colon (12,15,51). These particular conditions include different pH values in the colon and colon specific microflora and microbial enzymes (52).

1.1.2.1. pH-dependent Drug Delivery System

Currently, commercial products for local drug delivery targeting the colon are mostly based on pH changes within the GI tract (53). The fact that pH based systems enable the incorporation of a higher level of drug when compared to other delivery systems, such as microbially triggered systems, may have contributed to that (12,54). Furthermore, pH-dependent systems were found to be more effective than any other colon delivery system as far as targeting colon is concerned, which has been supported by numerous clinical studies (53). However, high intra and inter-individuals GI pH variability and lack of appropriate coating material dissolving at the desired pH of the colon has caused controversy about pH-dependent systems usefulness for the intended purpose (55,56).

In order to keep physical and chemical integrity of pH-dependent dosage forms in stomach and small intestine, the most commonly used coating polymers are derivatives of acrylic acid and cellulose (Table 1) (52,57). These polymers contain weakly acidic groups in their polymeric backbones that prevents their dissolution at low pH, since acidic groups will be protonated and, consequently, unionized. When they are exposed to a solution with higher pH values and a different ionic composition, their charge density changes leading to dissolution of the polyacidic polymers. Hence, drug release occurs with a pH sensitive release rate (57).

However, pH-response of polyacidic polymers can be altered by using neutral comonomers, such as methyl methacrylate (58). These comonomers modify polymer chain hydrophobicity leading to different pH-sensitive behaviour. In addition, it was also described that the presence of poly (methacrylic acid) (PMA) concomitantly with poly(ethylene glycol) (PEG) induces particular pH-sensitive properties (59). At low pH, the ether oxygen of PEG establishes hydrogen bonds with the acidic protons of the carboxyl groups of PMA. Such complexation leads to the shrinkage of the coating. When

pH levels increase and the PMA carboxyl groups ionize, decomplexation occurs leading to dissolution of the coating.

Table 1. Coating polymers utilized in development of pH-dependent drug delivery system for colonic delivery. [Adapted from (52,60)]

Polymers	Optimum pH for dissolution
Polyvinyl acetate phthalate (PVAP) (Coateric®)	5.0
Cellulose acetate trimellitate (CAT)	5.5
Hydroxypropyl methylcellulose phthalate (HPMCP)	
HP-50	≥ 5.0
HP-55 and HP-55S	≥ 5.5
Hydroxypropylmethylcellulose acetate succinate (HPMCAS)	
LF Grade	≥ 5.5
MF Grade	≥ 6.0
HF Grade	≥ 6.8
Methacrylic acid copolymer, Type C (Eudragit® L100-55) Methacrylic acid copolymer dispersion (Eudragit® L30D-55)	≥ 5.5
Methacrylic acid copolymer, Type A (Eudragit® L-100 and Eudragit® L12,5)	≥ 6.0
Cellulose acetate phthalate (CAP) (Aquateric®)	6.0
Methacrylic acid copolymer, Type B (Eudragit® S-100 and Eudragit® S12,5)	≥ 7.0
Eudragit® FS30D	≥ 7.0
Shellac (MarCoat 125 & 125N)	7.0

Although methacrylic acid copolymers are widely used as coating polymers, none of them is capable of initiating drug release at pH 6.5 (table 1), which is the standard pH value for proper drug delivery to the colon (55). To overcome this problem, associations of Eudragit S-100 with either Eudragit L100-55 or Eudragit L-100 were developed in various ratios. In this approach, the dissolution mechanism of the coating starts with pore (and/or weak points) formation in the film, due to faster solubilization of Eudragit L100-55/ Eudragit L-100 than Eudragit S-100. This leads to the formation of channels in the solid dosage forms, allowing penetration of the dissolution media, which results in a faster drug dissolution (55). In this way, it is possible to reduce the value of pH required to cause drug release without the need for the development of further coating polymers.

1.1.2.2. Microbially triggered systems

Microbially triggered drug delivery systems to the colon is possible due to a more diverse and abundant flora present in this section of GI tract, relatively to the other parts (28).

Human colon is mainly colonized by *Bacteroids, Bifidobacteria, Eubacteria, Enterobacteria and Enterococci* (61). Due to colon characteristic anaerobic microbiota and its distinct enzymatic secretions, two groups of site-specific drug delivery were developed, the polysaccharide based delivery system and the prodrug based delivery system (Figure 5).

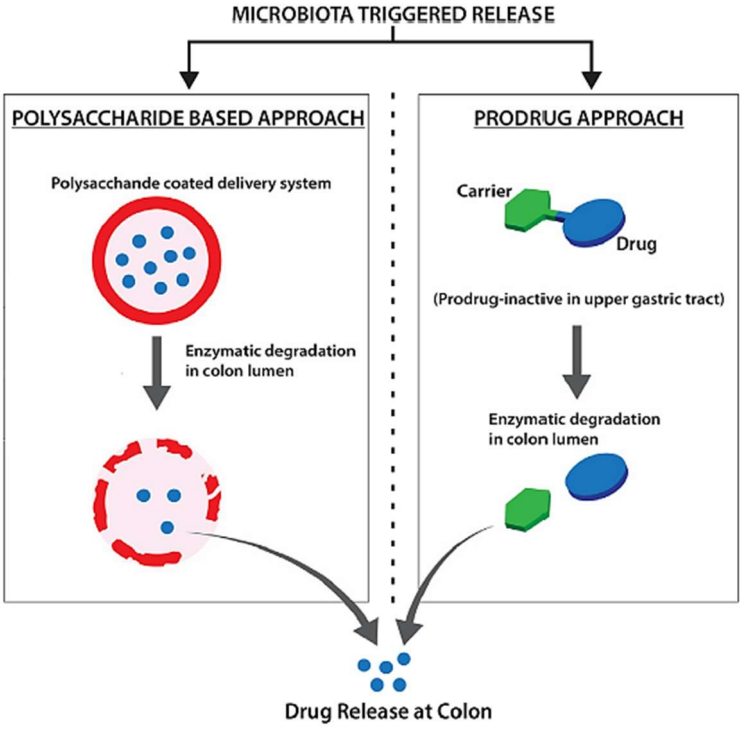


Figure 5. Differences between polysaccharide based delivery system and prodrug based delivery system. Adapted from (15)

The polysaccharide based drug delivery system arose from the fact that colon natural environment contains polysaccharidases in sufficient quantity to be exploited in targeting of drugs. Polysaccharidases are bacterial enzymes capable of degrading polysaccharides such as pectin, guar gum, amylose, inulin, dextran, chitosan and chondroitin sulphate (62). In this approach, dosage forms are coated with polysaccharides which only suffer enzymatic breakdown when they reach the colon, resulting in drug release. Polysaccharides as coating materials present several benefits. They have a predictable degradation pattern, which results in a consistent drug release. They are capable of hydrating and swelling, creating a barrier and preventing drug diffusion in the upper GI tract. Furthermore, some have high stability while expose to different temperatures, high biodegradability and low toxicity (63).

Regarding prodrug delivery system, this approach is based on a pharmacologically active drug covalently conjugated to a carrier, which requires enzymatic transformation *in vivo* to release the active drug (64). The type of linkage determines the conversion of prodrugs into active drugs. For colonic delivery, some

secreted enzymes of the GI tract microbiota, such as azoreductases, glucuronidases, nitroreductases, glycosidases, are exploited. The degradation capability of the linkages with such enzymes in that limited area of GI tract provides specificity to this drug delivery system (15). Azo conjugates are amongst the most extensively used linkages for colon drug delivery. These linkages have high thermal, chemical and photochemical stability (65). Other linkages susceptible to bacterial metabolism where the drug is attached to hydrophobic moieties as amino acids, glucuronic acids, glucose *etc*. have also been studied for colon delivery. However, the prodrug approach is not very versatile since its formulation depends upon appropriate functional groups on the drug moiety to conjugate. In addition to this drawback, the environmental conditions may need to be taken into consideration since they have an influence not only on the behaviour of the prodrug based delivery system but also on polysaccharide based delivery system. As explained in chapter 1.1., conditions such as microbiota enzymatic secretions changes in disease, gut infections and diet style may alter the specific degradation of the microbially triggered systems, leading to an imprecise release in the colon (15).

1.2. Aims of the Thesis

The aim of this study was the evaluation of mesalazine release of different marketed medicines, encompassing colonic drug delivery systems, in different pH media with different time intervals. These tests aim to mimic GI tract characteristics in order to predict *in vivo* drug release of such delivery systems.

To this end, the study was divided in two stages: a) investigation of the release rate of a drug present in colonic drug delivery systems in media containing similar pH values to the ones found in the GI tract; b) understanding the effect of exposition of dosage forms at low pH values, with the likely embedding of acidic entities on the hypothetical delay on the drug release rate.

Time-dependent and pH-dependent dosage forms containing mesalazine as active pharmaceutical ingredient (API) were selected for this study. All medicines studied are generally considered for the treatment of IBD, such as CD or UC, where inflammation is mainly localized in more distal regions of GI tract.

Chapter 2 – Materials and Methods

2.1. Materials

Table 2. Colonic drug delivery systems used in the study

Product	Dosage form	Manufacturer	Polymer type
Asacol	Coated tablet	Tillots Pharma GmbH, Rheinfelden, Germany	Eudragit S
Claversal	Coated tablet	Recordati Pharma GmbH, Ulm, Germany	Eudragit L/ S
	Pellets	Recordati Pharma GmbH, Ulm, Germany	Eudragit L 100-55/ S
Mezavant	Coated tablet	Shire, Berlin, Germany	Eudragit L/ S
Salofalk	Coated tablet	Dr. Falk Pharma GmbH, Freiburg, Germany	Eudragit L/ S
Pentasa	Immediate-release tablet containing coated granules	Ferring S.A.S., Gentillly, France	EC
	Coated granules	Ferring S.A.S., Gentillly, France	EC

All other chemicals were analytical grade or equivalent and purchased commercially.

2.2. Methods

2.2.1. Drug release experiments and sampling analysis

Dissolution tests, to assess the release of the drug, were run in triplicate for all the medicines except for those at pH 1 that were run in duplicate. All drug release experiments were performed according to the EP 9 (paddle method, rotating at 50 rpm, in dissolution media at 37±0.5 ºC, Vankel VK 7010, Vankel Industries, Edison, NJ, USA). The results were expressed as mean percentage (± SD) dissolved at the given sampling time.

2.2.1.1. Release tests in single media

Experiments were performed for all the medicines using different values of pH (pH 1 ± 0.05, 4.5 ± 0.05, 6.8 ± 0.05 and 7.4 ± 0.05). Vessels whose media is at pH 1 ± 0.05

contain 500 mL of hydrochloric acid (HCl) 0.1 N. Vessels at pH 4.5 ± 0.05 contain 500 mL of HCl 0.1 N and 112 mL of tribasic sodium phosphate 0.2 M. Vessels containing media at pH 6.8 ± 0.05 comprise 500 mL of HCl 0.1 N and 150 mL of tribasic sodium phosphate 0.2 M. Vessels at pH 7.4 ± 0.05 contain 500 mL of HCl 0.1 N and 180 mL of tribasic sodium phosphate 0.2 M. The whole test lasted for 24 h.

2.2.1.2. Release tests in a physiological-based pH gradient

Experiments started with 500 mL 0.1 N HCl (pH 1 ± 0.05). After 2 h, the pH was changed to 4.5 ± 0.05 by addition of 112 mL of tribasic sodium phosphate 0.2 M. Half an h later, the pH was adjusted to 6.8 ± 0.05 by adding 40 mL of tribasic sodium phosphate 0.2 M. After 3h, the pH was changed again to set pH 7.4 ± 0.05 by adding 50 mL of tribasic sodium phosphate 0.2 M and the experiment was continued until 24h.

2.2.1.3. Release tests in a physiological-based pH gradient with different residence times in pH 1

Experiments were performed with the same operative settings as in the previous experiment, except for the residence time of the medicines at pH 1. Instead of changing the media after 2h in 0.1 N HCl, the residence time was altered to 0.5h or 4h in 0.1 N HCl (n=3).

2.2.1.4. Sampling analysis

All samples were analysed at 260 nm (for pH 1 and 4.5) or at 272 nm (for pH 6.8 and 7.4) by UV (ultraviolet)-spectrophotometer. All samples were also measured at 400 nm to remove the background noise. The percentage of drug released was calculated using a standard curve ($R^2 \geq 0.999$) of appropriate standard solutions of mesalazine in the tested media. This spectroscopic method is regarded as simple, sensitive, selective, accurate, precise and economical in the determination of mesalazine (66).

2.2.2. Media uptake and dry mass loss measurements

The tablets were weighted [dry mass (0)] and then separately placed into 1000 mL plastic containers filled with 500 ml 0.1 N HCl, followed by horizontally shaking (GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany) at 37 ± 0.5 ºC and with a stirring speed of 80 rpm. In the first h, three pre-determined tablets were withdrawn, accurately weighed [wet mass (t)] and dried to constant weight at 60 ± 0.5 ºC [dry mass (t)]. The

same procedure occurred after 2 h in 0.1 N HCl, after 2h in 0.1 N HCl and 0.5 h in pH 4.5 (plastic containers filled with 500 mL of 0.1 N HCl and 112 mL of 0.2 M tribasic sodium phosphate), after 2h in 0.1 HCl, 0.5 h in pH 4.5 and 1h in pH 6.8 (plastic containers filled with 500 mL of 0.1 N HCl and 150 mL of 0.2 M tribasic sodium phosphate) and after 2h in 0.1 HCl, 0.5h in pH 4.5 and 2 h in pH 6.8. The media content (%) and dry mass loss (%) at the time t was calculated as follows:

$$\text{media uptake } (\%)(t) = \frac{\text{wet mass } (t) - \text{dry mass } (t)}{\text{wet mass } (t)} \times 100$$

$$\text{dry mass loss } (\%)(t) = \frac{\text{dry mass } (0) - \text{dry mass } (t)}{\text{dry mass } (0)} \times 100$$

Chapter 3 - Results

3.1. Investigation of the effect of pH on pH-dependent and time-dependent dosage forms

A larger understanding of drug release profile of these medicines not only allows to select the medicine that better suits each patient, but also helps to improve drug effectiveness (67). Thus, the insight of colonic drug delivery systems release patterns becomes of great importance.

In order to understand how several colonic drug delivery systems behave in the GI tract, a simulation of such delivery systems was performed, firstly, in single media presenting pH values similar to those found in GI tract (Figure 6).

The experiments performed with Asacol 400 mg and Asacol 800 mg revealed that it was necessary to reach pH values of 6.8 or higher for drug release started to occur. However, at pH 6.8, the drug release pattern of these two medicines was not the same. Asacol 800 mg required approximately 1 h longer to start the release of the drug when compared to Asacol 400 mg. Furthermore, it was necessary approximately 7 h for Asacol 400 mg and about 12 h for Asacol 800 mg to reach a complete mesalazine release at pH 6.8. At pH 7.4, both medicines presented immediate mesalazine release and both reached 100% of drug release in 6 h, approximately.

Regarding Mezavant 1200 mg, no significant mesalazine release was obtained through experiments performed at pH 1 and, at pH 4.5, mesalazine release only started after approximately 8 h. After 3 h at pH 6.8, about 10% of mesalazine had been released and, after 5 h, approximately 30% of mesalazine had been released. The experiments performed at pH 7.4 revealed that, after 3 h, approximately 20% of mesalazine had been released and, after 5 h, about 50% of drug release had occurred.

Salofalk also exhibited no mesalazine release at pH 1 until approximately 10h. At pH 4.5, drug release started about 5 h. Considerable variability between individual tablets in their release characteristics was observed both at pH 1 and at pH 4.5. Salofalk demonstrated complete release after about 5 h of exposure at pH 6.8 and complete release after approximately 3.5 h of exposure at pH 7.4.

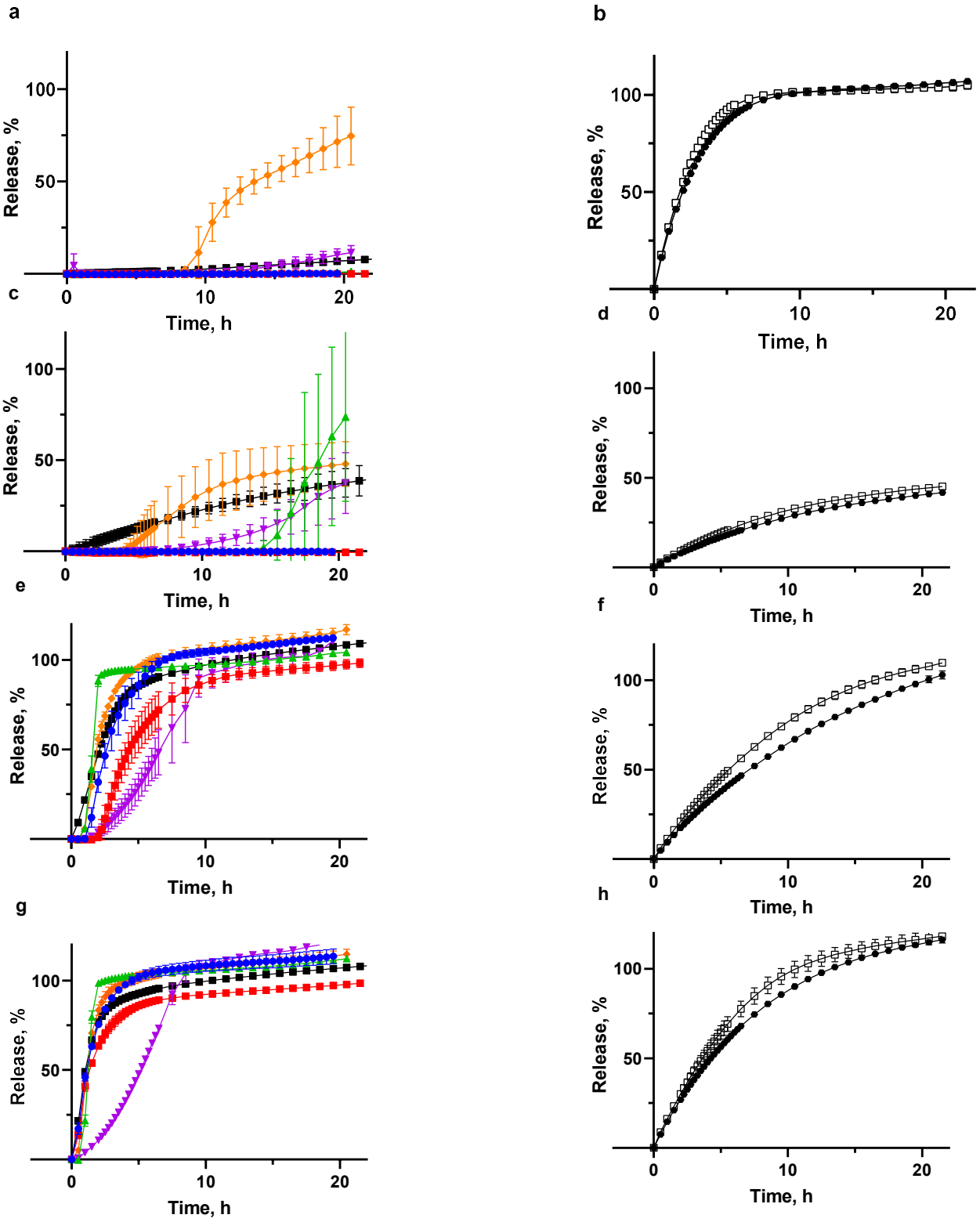


Figure 6. Dissolution profiles of different mesalazine dosage forms in single media simulating GI fluid. pH-dependent (**a, c, e, g**) (●, Asacol 400 mg; ■, Asacol 800 mg; ▲, Claversal 500 mg; ▼, Mezavant 1200 mg; ♦, Salofalk 1000 mg; ■, Claversal pellets 1500 mg) and time-dependent dosage forms (**b, d, f, h**) (□, Pentasa granules 1000 mg; ⬣, Pentasa 1000 mg) were studied in media at pH 1 ± 0.05 (**a, b**), media at pH 4.5 ± 0.05 (**c, d**), media at pH 6.8 ± 0.05 (**e, f**) and media at pH 7.4 ± 0.05 (**g, h**). Data is expressed as mean ± SD.

The experiments performed with Claversal tablets 500 mg and Claversal pellets 1500 mg demonstrated no relevant drug release at pH 1. At pH 4.5, mesalazine release profile of both dosage forms was quite different. Claversal pellets 1500 mg showed a drug release profile similar to what is found in a sustained release. On the other hand, Claversal tablets 500 mg only initiated mesalazine release after approximately 15.5 h and there was a considerable variability across individual tablets. At pH 6.8, Claversal tablets 500 mg started mesalazine release after 1 h and drug release in Claversal pellets 1500 mg was initiated after half an hour. In Claversal tablets 500 mg approximately 100% of drug release was obtained after 2 h at pH 6.8. Regarding Claversal pellets 1500 mg, though mesalazine release had started immediately, about 100% of drug release occurred after 6 h at pH 6.8. Claversal tablets 500 mg initiated mesalazine release after 1 h in pH 7.4 and, after 2 h, about 100% of mesalazine had been released. In contrast, Claversal pellets started drug release after half an hour at pH 7.4 and a complete mesalazine occurred approximately after 4 h at pH 7.4. Although both Claversal 500 mg and Salofalk 1000 mg contain the same types of polymers, they did not demonstrate the same drug release profiles in neither pH values.

Drug release from both Pentasa dosage forms started immediately upon contact with all different media. From all the media containing different pH values, media at pH 1 led to a faster mesalazine release since, after 2 h, this was the only media able to induce 50% of drug release in both Pentasa dosage forms. It is also important to notice that, in all media, Pentasa granules 1000 mg drug release occurred in a quicker manner when compared to Pentasa tablets 1000 mg.

After this first approach in a single media, release tests were performed based in a physiological pH gradient. In this way, it was intended to simulate the passage of such medicines in the GI tract (Figure 7). No medicine exhibited mesalazine release before reaching pH 6.8, except time-dependent dosage forms. Dissolution behaviour was similar between the dosage forms of each medicine.

Regarding experiments performed with Asacol 400 mg and Asacol 800 mg, Asacol 400 mg took approximately 2 h to start drug release when Asacol 800 mg took about 3 h. This contrasts with results obtained in dissolution tests performed in single media, where Asacol 400 mg took about one h and a half to initiate mesalazine release and Asacol 800 mg took approximately 2 h.

The Mezavant started its mesalazine release after about 1 h, at pH 6.8 and, after approximately 3 h 10% of mesalazine had been released. These results are in concordance with those presented in Figure 6e.

The dissolution profile of Salofalk 1000 mg revealed that, at pH 6.8, this dosage form initiated mesalazine release immediately. This is not coincident to the results found in Figure 6e where it was necessary about 1 h for Salofalk 1000 mg to start drug release.

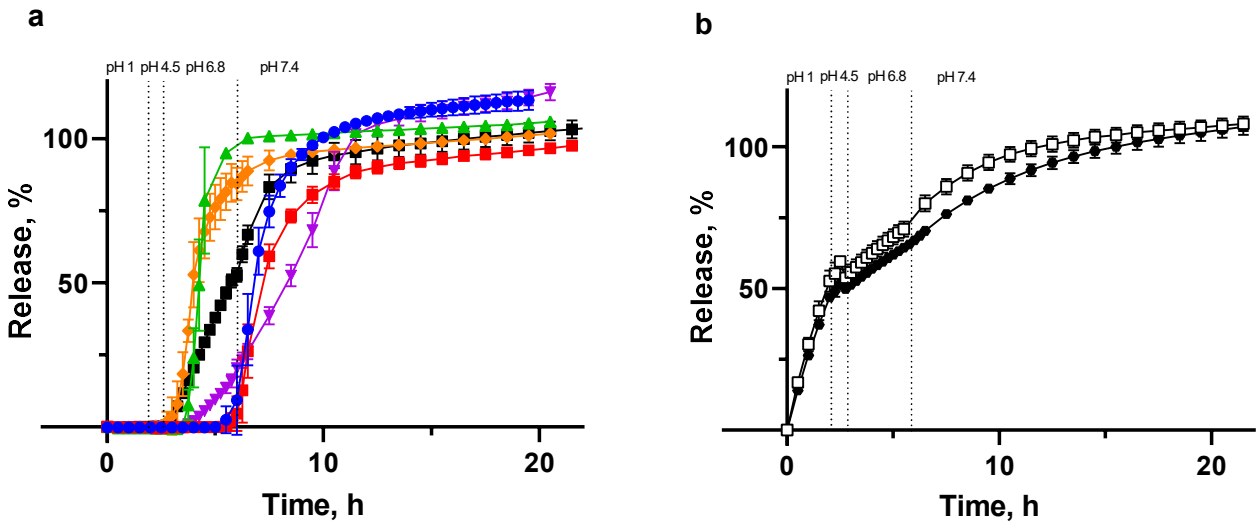


Figure 7. Dissolution behaviour of different mesalazine dosage forms in a pH gradient media simulating GI passage. pH-dependent (**a**) (●, Asacol 400 mg; ■, Asacol 800 mg; ▲, Claversal 500 mg; ▼, Mezavant 1200 mg; ♦, Salofalk 1000 mg; ■, Claversal pellets 1500 mg) and time-dependent dosage forms (**b**) (□, Pentasa granules 1000 mg; ⬣, Pentasa 1000 mg) were studied in media at pH 1 ± 0.05, at pH 4.5 ± 0.05, at pH 6.8 ± 0.05 and at pH 7.4 ± 0.05 with a continuous passage in the Paddle apparatus. Data is expressed as mean ± SD.

The experiments performed with Claversal tablets 500 mg and Claversal pellets 1500 mg showed quite different results from one another. Claversal tablets 500 mg initiated its mesalazine release after 1 h, at pH 6.8. Claversal pellets 1500 mg started its drug release after approximately 15 min at pH 6.8. These results are according to those found in release tests in single media (Figure 6e). However, it took about 3 h for Claversal tablets 500 mg to achieve approximately 100% of mesalazine release and about 6.5 h for Claversal pellets 1500 mg to achieve the same result after these two dosage forms had initiated drug release. This is not in agreement with the results presented in Figure 6e where, after starting drug release, Claversal tablets 500 mg achieved around 100% mesalazine release after 1 h and for Claversal pellets 1500 mg it took about 6 h. Furthermore, though Claversal tablets 500 mg and Salofalk 1000 mg contain the same type of polymers, they did not present the same mesalazine release profiles.

Results from both Pentasa dosage forms showed that mesalazine release started immediately and to a greater extent at pH 1. After 2 h, at pH 1, both dosage forms had released approximately 50% of mesalazine. This is in accordance with the results

presented in Figure 6b. Moreover, it is also important to highlight that Pentasa granules 1000 mg developed a faster mesalazine release in comparison to Pentasa tablets 1000 mg. The same results were obtained in release tests in single media (Figure 6b, 6d, 6f and 6h).

Finally, the experiments on drug release, following media uptake and dry mass loss were performed for Asacol 400 mg and Asacol 800 mg tablets. This method aimed to understand whether the dissolution profiles observed in Figure 6 and Figure 7 were linked to a media uptake by these medicines and if this media uptake resulted in the loss of mass by these medicines (Figure 8).

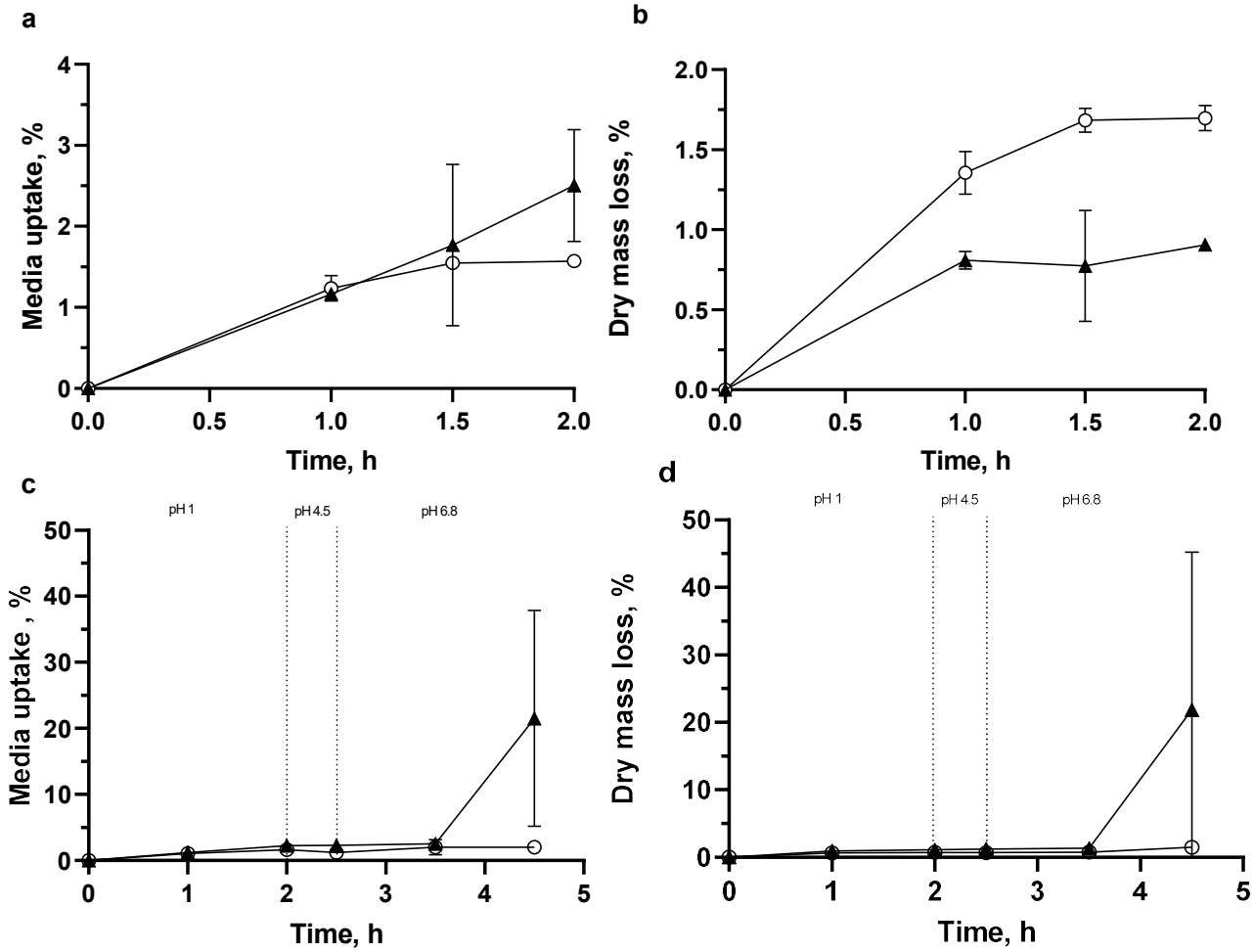


Figure 8. Effect of media pH on media uptake (a, c) and dry mass loss (b, d) of pH-dependent dosage forms. pH-dependent dosage forms (▲, Asacol 400; ○, Asacol 800 mg) were studied in media at pH 6.8 ± 0.05 (**a, b**) and with a continuous passage in media at pH 1 ± 0.05, at pH 4.5 ± 0.05 and at pH 6.8 ± 0.05 (**c, d**). Data is expressed as mean ± SD.

Experiments performed with Asacol 400 mg and Asacol 800 mg at pH 6.8 revealed that Asacol 400 mg tended to uptake media to a greater extent than Asacol 800 mg (Figure 8a). However, that did not result in a higher dry mass loss by the Asacol 400 mg. In fact, Figure 8b shows that dry mass loss by both medicines did not increase significantly after 1 h, at pH 6.8.

Asacol 400 mg, when subjected to a continuous passage through media at pH 1, pH 4.5 and pH 6.8 (Figure 8c), exhibited the same amount of media uptake after 3.5 h as after 2 h at a fixed pH 6.8 (Figure 8a). Nonetheless, for the same time intervals, the dry mass loss by Asacol 400 mg was higher when exposed to different media than when it was exposed only to pH 6.8 (Figure 8d). For Asacol 800 mg a higher media uptake was observed after 3.5 h in a pH changing media than after 2 h, at pH 6.8 (Figure 8c). However, in contrast to what was seen in Asacol 400 mg, that resulted in a greater dry mass loss by Asacol 800 mg subjected to 2 h, at pH 6.8 than Asacol 800 mg exposed to a continuous passage in media at pH 1, pH 4.5 and pH 6.8 for 3.5 h (Figure 8d).

Furthermore, as seen in Figure 8c and 8d, an increased amount of media uptake and dry mass loss occurred after Asacol 400 mg was exposed to pH 6.8 for 2 h. This occurred due to the disintegration of one of the tablets (Figure 9a) while the other two maintained their integrity. Nevertheless, though it was observed that one tablet also started its disintegration after 2 h, at pH 6.8 in dissolution tests (Figure 9b), the dissolution behaviour of Asacol 400 mg in a pH gradient media simulating GI passage did not demonstrated it (Figure 7a).

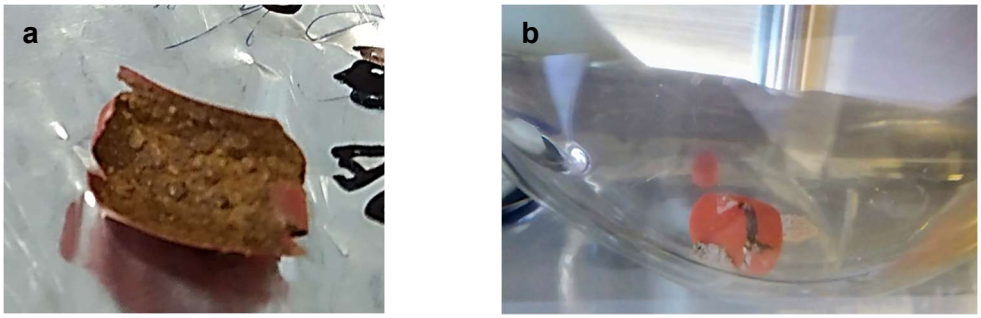


Figure 9. Tablet disintegration after 4.5 h, at physiological-based pH gradient media by media uptake and dry mass loss method (a) and by dissolution tests (b).

3.2. Effect of residence time at pH 1 on Asacol 400 mg tablets

In order to understand if the residence time in pH 1 has an influence on the release profile of Asacol 400 mg, release tests in a physiological-based pH gradient were performed varying residence times at pH 1 (Figure 9).

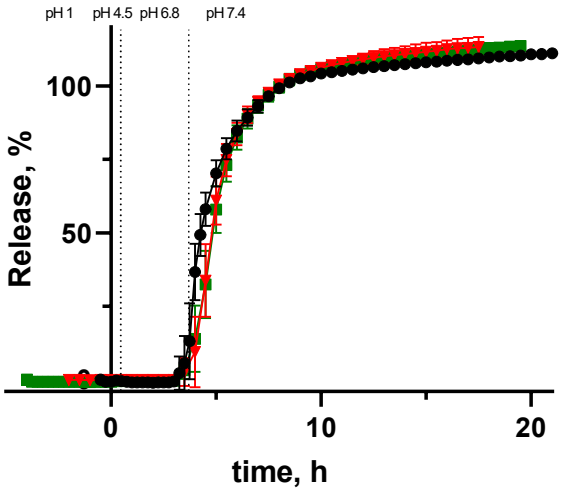


Figure 10. Dissolution behaviour of Asacol 400 mg with a pre-treatment at pH 1 for 0.5 h (●), for 2 h (▼) and for 4 h (■). Data is expressed as mean ± SD.

Release experiments showed slight differences in drug release as far as pre-treatments at pH 1 for 0.5 h, for 2 h and for 4 h are concerned. Regarding the pre-treatment at pH 1 for 0.5 h, after exposure to pH 6.8, it took approximately 2.5 h for Asacol 400 mg to initiate mesalazine release and 3 h to reach about 36% mesalazine release. For Asacol 400 mg experiments with a residence time at pH 1 of 2 h, mesalazine release began after 2.5 h, at pH 6.8 and after 3 h approximately 33% of mesalazine had been released. Lastly, Asacol 400 mg exposed to pH 1 for 4 h initiate mesalazine release after 2.75 h, at pH 6.8 and 15 minutes later about 14% of mesalazine had been released. However, these release profiles seem to be different from the dissolution behaviour of Asacol 400 mg in single media at pH 6.8 (Figure 6e). In these conditions, Asacol 400 mg initiated mesalazine release after 1.5 h at pH 6.8 and, after 3 h, these tablets exhibited about 75% mesalazine release.

Chapter 4 - Discussion

At the turn of 21st century, the IBD, including UC and CD, became a global disease with special incidence in industrialized countries. Nowadays, it is estimated that over 1.5 million people in North America and 2 million people in Europe suffer from the disease (68,69). However, the incidence and prevalence rates of IBD have demonstrated a tendency to increase in Asia and Africa (70). Therefore, IBD has become a disease with a broad socioeconomic burden (68,70,71), highlighting the importance for a better understanding not only of the disease itself but also of the therapy to which patients are submitted.

Mesalazine is the first-line treatment for patients with mild to moderate UC (72). To date, numerous single units and multiparticulate dosage forms containing mesalazine have been registered for the oral therapy of UC. Considering that this disease is a chronic remitting active inflammatory pathology and that patients will probably need lifelong therapy (73), it is of major importance the study of these medicines in order to characterize with high accuracy how they will behave *in vivo*. This will allow us to understand which medicine best fits each patient, improving drug effectiveness and reducing adverse drug reactions.

In recent years, drug release and availability at the site of action have been regarded as the major players determining clinical response for locally-acting GI medicines, such as mesalazine medicines (74). Thus, it is crucial to evaluate the dosage form performance for these medicines since drug release and dissolution are the main factors of the rate and extent to which the API is delivered to the site of action (73,75). Regardless of administering a monolithic or a multiparticulate dosage form, the evaluation of these parameters should provide a predictable and robust release profile ensuring a targeted mesalazine release to guarantee maximum efficacy (73).

Although clinical trials are required to prove therapeutic efficacy, a reduced quantity of comparative trials have been conducted with equivalent mesalazine doses to verify whether any of the current medicines available in the market is superior in the treatment of UC (76,77). Recently, various alternatives, including *in vitro* tests, are capable of replicating drug release and availability at the site of action by attempting to simulate typical intraluminal pH conditions in the human GI tract. In some of these tests, the application of a small set of compendial media to simulate the passage through stomach, small intestine and proximal colon is included (75–77). Other tests are considered more dynamic, reflecting the conditions relevant for a more accurate *in vivo*

drug release (73,81–85). These latter tests rely on the use of biorelevant dissolution media, where the media alone are able to mimic the different parts of the GI tract in a fasted and fed state (86).

Thus, when developing *in vitro* tests to evaluate mesalazine dosage forms drug release, it is important to take into consideration physiological parameters that have an influence on drug release in the GI tract of the patient. Some of these parameters include the pH conditions, the fluid volumes and compositions, and the transit times through different human GI tract sections (73).

In this study, the pH conditions and the transit times through GI tract were considered for the design of the *in vitro* tests. The study aims to predict site and extent of *in vivo* drug release of a selection of mesalazine medicines available in the market. Results from such predictive *in vitro* drug release experiments should help to estimate the effective fraction of mesalazine dose available at different sites in the GI tract, assessing patient benefits and risks that could be presented with the administration of different types of monolithic and multiparticulate dosage forms.

First, in order to understand Asacol drug release, dissolution studies were performed. The results showed that Eudragit S only started its dissolution after media pH reach 6.8 and that the dissolution rate of Eudragit S was dependent on pH values since it took longer for Eudragit S to dissolve in pH 6.8 when compared to pH 7.4. This influence of media pH on the dissolution rate of Eudragit S in Asacol was also observed in the studies performed by Klein et al. (85) and Spencer et al. (87). In the latter study, 100 % of mesalazine was released from the Asacol tablets within 2 h, at pH 7.2, whereas complete release of mesalazine at pH 6.8 occurred only after approximately 5 h. It is hypothesized that this effect due to pH might be due to the fact that media with higher pH values contain a higher concentration of anionic groups, leading to a faster change in the charge of acidic groups of Eudragit S chain. This charge shift will cause the Eudragit S methacrylic groups to ionize, making them easier to dissolve in the medium where they are inserted. However, it was observed that, when Asacol tablets were previously exposed to acidic media, the release of mesalazine was delayed in time (Figure 7). To understand this variation, dissolution studies were performed with Asacol 400 mg varying the time at pH 1 (Figure 10). The results showed minimal difference between all pre-treatment times at pH 1. The only relevant difference occurred between the results with and without pH 1 pre-treatment. This leads to the hypothesis that Asacol may be incorporating acid into its structure when in contact with acidic media, delaying mesalazine release. Nevertheless, this acid incorporation is not time dependent. The media uptake and dry mass loss method did not present conclusive results to support this hypothesis (Figure 8). Furthermore, the results showed that, at pH 6.8 in both single

media dissolution tests and pH gradient dissolution tests, Asacol 400 mg had a higher mesalazine release rate than Asacol 800 mg. This may be due to the possibility that Asacol 800 mg may contain a thicker film-coat, since it is postulated that tablet film-coat thickness is a crucial characteristic for the onset of drug release (80,83,87). At pH 7.4, these differences in drug release were not observed because the media pH was quite higher than the minimum pH required for polymer dissolution (85).

The Mezavant did not exhibit a relevant mesalazine release at pH 1 and pH 4.5 in single media dissolution tests and pH gradient dissolution tests (Figure 6). At pH 4.5, it would take 8 h for Mezavant to initiate mesalazine release and these conditions are not found in the GI tract. At pH 6.8, in both dissolution tests, Mezavant started its drug release after 1 h (Figure 6 and Figure 7). Compared to Asacol, there was an earlier onset of mesalazine release since Mezavant has in its composition a mixture of Eudragit L and Eudragit S. The mixture of these two different types of polymers decreases the pH required for polymer dissolution, resulting in earlier drug release. These results are in agreement with those found in Klein et al. (85) and Abinusawa et al. (80) for this dosage form in the same conditions. However, unlike Asacol, a burst mesalazine release did not occur. This prolonged drug release, characteristic of Mezavant, results from the fact that, inside the coating, mesalazine is embedded into a matrix structure comprising both hydrophilic and lipophilic excipients with extended release (53,85). Moreover, the results presented in Figure 6 lead to the hypothesis that media with a higher pH level tend to accelerate this extended release of Mezavant, since mesalazine release at pH 7.4 media is faster than drug release at pH 6.8 media. Furthermore, these differences in media pH level seem to have a slight influence in the onset of mesalazine release.

The experiments performed with Salofalk at pH 1 and pH 4.5 media showed that after approximately 10 h and 5 h, respectively, mesalazine release initiated (Figure 6). In both dissolution tests, considerable variability between individual tablets was observed. Similar results were obtained by Stolk et al. (88) for pH 1. Nonetheless, a more consistent release occurred at pH 6.8. At this pH, the Salofalk started mesalazine release after 1 h in the single media dissolution test and almost immediately in the pH gradient dissolution test. The experiments performed by Klein et al. (81,85) and Abinusawa et al. (80) support the results obtained for Salofalk at pH 6.8 in single media dissolution test. Due to the results of pH-gradient dissolution test, it can be hypothesized that the contact of Salofalk with acidic media may had dissolved part of the polymer, leading to a faster mesalazine release when this dosage form reached the media at pH 6.8. Since the Salofalk film-coating contains a mixture of Eudragit L and Eudragit S, the dissolution of the polymer is expected to occur in media whose pH is between 6 and 7. Once polymer dissolution occurs, a burst-like drug release can be observed for Salofalk since there is

not any other excipient in the formulation retaining the API. However, even though Mezavant contains the same type of polymers in its film-coating, mesalazine release did not occur at the same time. This may result from the fact that the tablets have films with different thicknesses (81) or because the ratio Eudragit L/ Eudragit S is not equal (55). However, it is necessary to perform other studies to confirm this hypothesis. In the single media dissolution study performed at pH 7.4, Salofalk initiated its drug release earlier and faster than the same dosage form in media at pH 6.8. This occurred because hydrogen ion concentration is lower at pH 7.4, ionizing faster the film-coating polymer of Salofalk, which led to a faster drug release.

Regarding Claversal tablets, no drug release was observed in both single media dissolution test and pH-gradient dissolution test at pH 1 (Figure 6 and Figure 7). However, after about 15 h, at pH 4.5, Claversal tablets initiated its mesalazine release with high tablet-to-tablet variability. Nevertheless, drug release after 15 h, at pH 4.5 is an unlikely *in vivo* scenario since these conditions are not found in the GI tract. At pH 6.8, in both dissolution tests, Claversal tablets initiated its drug release after about 1 h. Since Claversal tablets and Salofalk contain the same type of polymers, it would be expected that both present similar release profiles. However, at pH 6.8 in single media dissolution tests, the burst-like drug release of Claversal tablets occurred earlier and to a higher extent. On the other hand, in pH-gradient dissolution test, Salofalk started to release mesalazine 1 h earlier than Claversal. As explained before, these distinct release profiles may be due to differences in film-coating thickness, Eudragit L/Eudragit S ratio and to different environments to which these tablets are submitted, adding more variables to keep in mind when trying to predict the drug release of these dosage forms into the GI tract. These differences between Claversal tablets and Salofalk were also described in the studies performed by Klein et al. (81,83,85) and by Karkossa et al. (73). In single media dissolution study at pH 7.4, these differences between these two medicines were not so clear since this pH level was quite higher than the minimum pH required for polymer dissolution.

The results of Claversal pellets in single media dissolution tests showed that dissolution of the polymer initiated after this medicine is in contact with pH 4.5. However, the mesalazine release rate was not so fast as in pH 6.8 and pH 7.4 media (Figure 6). In the pH-dependent dissolution test, the onset of mesalazine at pH 4.5 was not observed because the dosage form was only in contact with that media for 30 min. The earlier drug release of Claversal pellets at pH 4.5 in single media dissolution test, relatively to the other pH-dependent dosage forms, is due to the fact that Claversal pellets film-coating contains a mixture of Eudragit L 100-55 and Eudragit S. Since Eudragit L 100-55 starts its dissolution at pH 5.5, this will lead to an earlier drug release onset when compared to

the other medicines referred above. In addition, Claversal pellets, at pH 4.5, presented a drug release profile similar to an extended release. This release profile occured probably because pH 4.5 is not high enough to induce a fast dissolution of the pellets polymer, resulting in the fact that some pellets released mesalazine earlier than the others. Comparable data were obtained in the studies performed by Karkossa et al. (73). Furthermore, one should keep in mind that, in these release experiments, the multiparticulate dosage forms were treated as monolithic dosage forms. However, it is stated that multiparticulate dosage forms are likely to spread and move through different parts of the GI lumen in discrete portions (89,90). Thus, it should be noticed that the amount of drug available at the different intraluminal sites cannot be 100% represented by this study.

Pentasa granules showed a similar drug release profile as the Pentasa tablets, in all dissolution tests performed. This is to be expected since Pentasa tablets, when in contact with an aqueous environment, fall apart into EC-coated granules. The results of Figure 7b are explained by the fact that, as explained above, EC is not pH-dependent nor depends on ionic strength. The mesalazine release in these two dosage forms will depend on the erosion rate of EC, exhibiting a sustained drug release. The mesalazine in the outer parts of the granules dissolves quickly in the acid solvent, decreasing gradually due to time needed by the inner mesalazine to diffuse to the surface. Similar results were found in other studies (73,80,81,83,84,88,91,92). However, the results in Figure 6 suggested that there is a relationship between the value of the pH and the release of mesalazine. Since EC is pH-independent, the variable that might be operating in these release profiles is mesalazine solubility. It is reported that mesalazine solubility is higher at pH values lower than 2 and higher than 5.5 (73,93). Therefore, it is expected that mesalazine release rate is higher at pH 1 and pH 7.8. However, drug release rate at pH 1 is faster than at pH 7.8. This may be explained because, when mesalazine is released in the dissolution medium, the medium pH decreases since mesalazine itself is an acidic molecule. This decrease in the pH dissolution medium will reduce mesalazine solubility, leading to a slower dissolution of mesalazine in alkaline media. This characteristic may be useful to control mesalazine release in the alkaline pH of the colon. Due to this relationship between mesalazine and media pH, some authors (94) consider Pentasa dosage forms not only time-dependent but also pH-dependent. Thus, these data showed that gastric transit time is a factor of major importance for mesalazine release *in vivo* since, in case of a longer residence time in the stomach, a small amount of mesalazine will be released in the colon.

Chapter 5 – Final Comments

5.1. Conclusions

The data obtained in the framework of this work elucidate the oral colon-specific delivery of the intact drug substance of several commercially available medicines. This is a strategy to increase the concentration of the drug locally and to reduce systemic toxicity due to a decrease of absorption in higher parts of the GI tract. Although the studies performed do not include all GI factors responsible for changes in drug release, the results suggest that pH media has an influence in all tested formulations, even those which are not considered pH dependent.

For both Asacol tablets, the results showed that it is expected that mesalazine release will occur in the distal part of the small intestine and ascending colon since these dosage forms contain only Eudragit S. In addition, it can be hypothesized that exposure to acidic media may delay drug release. Due to a possibly thicker film-coat of Asacol 800 mg, it is expected a lower mesalazine release rate compared to Asacol 400 mg.

The results of the experiments performed with Mezavant suggest that the onset of mesalazine release in this medicine occurs in the proximal part of the small intestine, but mesalazine release may be extended until Mezavant reach the colon. This is due to the fact that Mezavant contains extended release excipients in its structure. Furthermore, this extended release seemed to be pH-dependent.

Salofalk is likely to release mesalazine close to the proximal portion of the small intestine due to its mixture of Eudragit L and Eudragit S. Moreover, the contact of this medicine with acidic properties seem to cause the dissolution of part of the polymers, leading to a faster mesalazine release.

Regarding Claversal tablets, an earlier drug release occurred when compared to Salofalk. It is hypothesized that these two distinct release profiles are due to differences in film-coat thickness or because Eudragit L/ Eudragit S ratio is different.

The onset of mesalazine release in Claversal pellets occurred earlier than in the other pH-dependent medicines, due to its mixture of Eudragit L 100-55 and Eudragit S.

For both Pentasa dosage forms, their extended release is dependent on the erosion of EC. However, mesalazine solubility seem to be the factor that causes different release profiles in the different media. A higher solubility at pH 1 induces higher mesalazine release rates. Thus, the mesalazine release in these dosage forms will be

dependent on their residence time in the stomach. Longer gastric residence times will lead to smaller amounts of mesalazine in the distal portion of the small intestine and colon.

5.2. Future Work

The next steps should focus on designing *in vitro* studies that mimic also other GI characteristics and *in vivo* studies to evaluate dosage forms pathway throughout the GI tract.

For *in vitro* studies, the presence of *Enterobacteria, Enterococci* and other microorganisms should be a factor to consider since it is reported that microbial flora of the colon is able to degrade some polymers, leading to drug release. Furthermore, different mechanical pressure throughout the GI tract should also be mimicked in *in vitro* studies. These differences in mechanical pressure have a significant impact on the release behaviour of some dosage forms since the mechanical pressure may induce the compaction of the medicines core matrix, which results in lower drug release rates.

Regarding *in vivo* studies, gamma-scintigraphy can be performed in healthy individuals and in individuals suffering from IBD, in the fed and fasted state. In this non-invasive technique, the locally - acting mesalazine medicines are attached to radioisotopes and the medicines are administered via oral route for emitting gamma rays. The emitted gamma radiation is captured by external gamma detectors to obtain computerized images (95). With gamma-scintigraphy technique, it is possible to assess *in vivo* disintegration times of the various dosage forms in the different parts of the GI tract. This way, a more complete understating of colonic drug delivery systems will be achieved.

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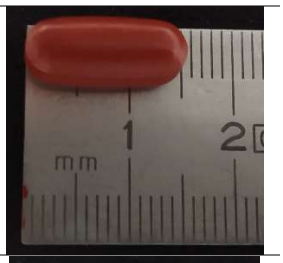
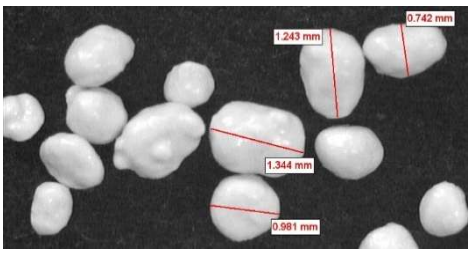
Annexes

Annex A: Characterization of the dosage forms - Tablets

Brand	Dosage (mg)	Weight (g)	Height (mm)	Length (mm)	Width (mm)	Picture
Asacol	400	0.5419	6	15	6	
Asacol	800	1.0849	7	17	8	
Claversal	500	0.8789	7	18	9	
Mezavant	1200	1.4046	7.5	21	10	
Salofalk	1000	1.3411	7	21	10	
Pentasa	1000	1.4996	7	20	11	

Annex B: Characterization of the dosage forms – Multiparticulate dosage forms

Brand	Dosage (mg)	Weight (g)	Height (mm)	Length (mm)	Diameter (mm)	Picture
Claversal	1500	2.9954	0.742	1.2935	0.981	
Pentasa	1000	1.4996	0.930	1.4685	0.835